

We claim:

1. A double-stranded DNA molecule, comprising an expression cassette containing a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell, wherein said molecule:
 - is in circular and supercoiled form,
 - lacks an origin of replication,
 - lacks a selection marker gene, and
 - comprises a sequence *attL* resulting from site-specific recombination between an *attB* and an *attP* sequence or between an *attB* derived sequence and an *attP* derived sequence, said region being located outside the expression cassette.
2. The molecule according to claim 1, further comprising a sequence that interacts specifically with an oligonucleotide to form a triple helix by hybridization.
3. The molecule according to claim 2, wherein the sequence that forms a triple helix comprises from 5 to 30 base pairs.
4. The molecule according to claim 2, wherein said molecule is MC3909, MC3948, or MC4009.
5. The molecule according to claim 4, wherein the sequence that forms a triple helix and the sequence *attL* are contiguous and are as set forth in SEQ ID NO: 12.
6. The molecule according to claim 1, further comprising an *mrs* sequence originating from a *par* locus of RK2.
7. The molecule according to claim 1, wherein the gene of interest is a nucleic acid coding for a therapeutic, vaccine, agricultural, or veterinary product.

8. The molecule according to claim 1, wherein said molecule is obtained by excision from a plasmid or chromosome by site-specific recombination.
9. A double-stranded DNA molecule, comprising an expression cassette containing a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell, wherein said molecule:
- is in circular and supercoiled form,
 - lacks an origin of replication,
 - lacks a selection marker gene, and
 - comprises a sequence *attB* resulting from site-specific recombination between an *attL* and an *attR* sequence or between an *attL* derived sequence and an *attR* derived sequence, said region being located outside the expression cassette.
10. The molecule according to claim 9, further comprising a sequence that interacts specifically with an oligonucleotide to form a triple helix by hybridization.
11. The molecule according to claim 10, wherein the sequence that forms a triple helix comprises from 5 to 30 base pairs.
12. The molecule according to claim 10, wherein said molecule is MC3955 or MC4007.
13. The molecule according to claim 12, wherein the sequence that forms a triple helix and the sequence *attB* are contiguous and are set forth in SEQ ID NO: 13.
14. The molecule according to claim 9, further comprising an *mrs* sequence originating from a *par* locus of RK2.
15. The molecule according to claim 9, wherein the gene of interest is a nucleic acid coding for a therapeutic, vaccine, agricultural, or veterinary product.

16. The molecule according to claim 9, wherein said molecule is obtained by excision from a plasmid or chromosome by site-specific recombination.
17. A plasmid comprising:
- a) a bacterial origin of replication and a selection marker gene; and
 - b) a polynucleotide comprising an expression cassette positioned between the *attB* sequence and the *attP* sequence of a bacteriophage lambda, P22, Φ 80, P1, or HP1, or of plasmid pSAM2 or between an *attB* derived sequence and an *attP* derived sequence, positioned in direct orientation, which recombine by site-specific recombination in the presence of an integrase, wherein said expression cassette comprises a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell, and wherein the origin of replication and the selection marker gene are located outside of said polynucleotide.
18. The plasmid according to claim 17 wherein said plasmid is pXL3909, pXL3948 or pXL4009.
19. The plasmid according to claim 17, wherein the *attB* and *attP* sequences which recombine by site-specific recombination are attachment sequences of bacteriophage lambda.
20. The plasmid according to claim 17, wherein the selection marker gene is a gene for kanamycin resistance or a tRNA suppressor (supPhe).
21. The plasmid according to claim 17, further comprising a sequence that interacts specifically with an oligonucleotide to form a triple helix by hybridization.
22. The plasmid according to claim 17, wherein the sequence that forms a triple helix comprises from 5 to 30 base pairs.

0931803-104901

23. A plasmid comprising an expression cassette positioned between attL and attR sequences of a bacteriophage lambda.

24. The plasmid according to claim 23, further comprising an origin of replication and a selection marker gene, wherein the origin of replication and selection marker gene are located outside said polynucleotide.

25. The plasmid according to claim 24, wherein said plasmid is pXL3955 or pXL4007.

26. The plasmid according to claim 24, wherein the selection marker gene is a gene for kanamycin resistance or a tRNA suppressor (supPhe).

27. The plasmid according to claim 23, further comprising a sequence that interacts specifically with an oligonucleotide to form a triple helix by hybridization.

28. A plasmid comprising:

(a) a bacterial origin of replication and a selection marker gene; and

(b) a polynucleotide comprising an expression cassette positioned between attL and attR sequences of a bacteriophage lambda, p22, Ø80, P1 or HP1, or of a plasmid pSAM2 or between an attL derived sequence and an attR derived sequence, positioned in direct orientation, which recombine by site-specific recombination in the presence of an integrase and an excisionase, wherein said expression cassette comprises a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell, and wherein said polynucleotide lacks an origin of replication and a selection marker gene.

29. A cultured recombinant cell comprising the recombinant DNA according to claim 17.

30. The cultured recombinant cell according to claim 29, wherein said cell is a bacterium.

31. The cultured recombinant cell according to claim 30, further comprising within its genome a gene coding for an integrase under the control of a plac UV5 promoter and designated *Escherichia coli* strain G6191.

32. The cultured recombinant cell according to claim 30, further comprising within its genome a gene coding for an integrase under the control of pBAD promoter, and which is designated *Escherichia coli* strain G6264.

33. The cultured recombinant cell according to Claim 30 further comprising within its genome an integrase gene and an excisionase gene under the control of the pBAD promoter, and which is designated *Escherichia coli* strain G6289.

34. A method for preparation of the DNA molecule according to claim 1, comprising culturing:

a) a host cell comprising a recombinant DNA comprising a nucleic acid consisting of an expression cassette positioned between an *attB* and an *attP* sequence or between an *attB* derived sequence and an *attP* derived sequence positioned respectively at 5' end and 3' end of the expression cassette, in direct orientation, which recombine by site-specific recombination in the presence of an integrase to form an *attL* sequence, and wherein the expression cassette comprises a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell; with

2) an integrase, whereby site-specific recombination occurs between the two sequences positioned in direct orientation.

35. The method according to claim 34, wherein the cultured host cell is brought into contact with the integrase by transforming or infecting the cultured host cell with a plasmid or a phage containing a gene for the integrase.
36. The method according to claim 35, wherein the cultured host cell is brought into contact with the recombinase by inducing expression of a gene coding for the integrase, wherein the gene is present in the host cell.
37. The method according to claim 36, wherein the host cell comprises within its genome an integrase gene, wherein expression of the integrase gene is regulated by a chemically-inducible promoter, and further wherein the cultured host cell is brought into contact with the integrase by culturing the host cell in the presence of a chemical inducer of the promoter, whereby expression of the integrase gene is induced.
38. The method according to claim 37, wherein the chemically-inducible promoter is pBAD.
39. The method according to claim 38, wherein the host cell is *Escherichia coli* strain G6264.
40. The method according to claim 39, wherein the chemically-inducible promoter is placUV5.
41. The method according to claim 40, wherein the host cell is *Escherichia coli* strain G6191 or G6191-lacI^{q1}.
42. A method for preparation of the DNA molecule according to claim 9, comprising culturing 1) a host cell comprising a recombinant DNA comprising a nucleic acid consisting of an expression cassette positioned between an *attR* and an *attL* sequence

or between an *attR* derived sequence and an *attL* derived sequence positioned in direct orientation, which recombine by site-specific recombination in the presence of an integrase and an excisionase to form an *attB* sequence, and wherein the expression cassette comprises a gene of interest under control of a transcription promoter and a transcription terminator active in a mammalian cell with 2) an integrase and an excisionase, whereby site-specific recombination occurs between the two sequences positioned in direct orientation.

43. The method according to claim 42, wherein the cultured host cell is brought into contact with the integrase and the excisionase by transforming or infecting the cultured host cell with a plasmid or a phage containing at a gene for at least one of the recombinase or the excisionase.

44. The method according to claim 43, wherein the cultured host cell is brought into contact with the integrase and the excisionase by inducing expression of a gene coding for the integrase and a gene coding for the excisionase, wherein both genes are present in the host cell.

45. The method according to claim 44, wherein the host cell comprises within its genome an integrase gene and an excisionase gene, wherein expression of said genes is regulated by a chemically-inducible promoter, and further wherein the cultured host cell is brought into contact with the integrase and the excisionase by culturing the host cell in the presence of a chemical inducer of the promoter, whereby expression of the recombinase gene and of the excisionase gene is induced.

46. The method according to claim 45, wherein the chemically-inducible promoter is pBAD.

47. The method according to claim 46, wherein the host cell is *Escherichia coli* strain G6289.

48. The method according to claim 45, wherein the host cell comprises a lysogenic phage integrated in its genome and wherein the lysogenic phage comprises at least one of the gene for the integrase and the gene for the excisionase.

49. The method according to claim 34, further comprising purifying a minicircle formed by the site-specific recombination.

50. The method according to claim 49, wherein the minicircle is purified by first contacting the minicircle with a first specific oligonucleotide that is grafted onto a first support, whereby a triple helix is formed by hybridization of said first specific oligonucleotide with a first specific sequence present in the recombinant DNA, but not in the minicircle and by second contacting the minicircle with a second specific oligonucleotide that is grafted onto a second support, whereby a triple helix is formed by hybridization of said second specific oligonucleotide with a second specific sequence present in the minicircle.

51. The method according to claim 49, wherein the minicircle is purified by first contacting the minicircle with a first specific oligonucleotide that is grafted onto a first support, whereby a triple helix is formed by hybridization of said first specific oligonucleotide with a first specific sequence present in the minicircle, eluting the minicircle, and by second contacting the eluted minicircle with a second specific oligonucleotide that is grafted onto a second support, whereby a triple helix is formed by hybridization of said second specific oligonucleotide with a second specific sequence present in the recombinant DNA, but not in the minicircle.

52. The method according to claim 42, further comprising purifying a minicircle formed by the site-specific recombination.

53. The method according to claim 52, wherein the minicircle is purified by first contacting the minicircle with a first specific oligonucleotide that is grafted onto a first support, whereby a triple helix is formed by hybridization of said first specific oligonucleotide with a first specific sequence present in the recombinant DNA, but not in the minicircle and by second contacting the minicircle with a second specific oligonucleotide that is grafted onto a second support, whereby a triple helix is formed by hybridization of said second specific oligonucleotide with a second specific sequence present in the minicircle.

54. The method according to claim 52, wherein the minicircle is purified by first contacting the minicircle with a first specific oligonucleotide that is grafted onto a first support, whereby a triple helix is formed by hybridization of said first specific oligonucleotide with a first specific sequence present in the minicircle, eluting the minicircle, and by second contacting the eluted minicircle with a second specific oligonucleotide that is grafted onto a second support, whereby a triple helix is formed by hybridization of said second specific oligonucleotide with a second specific sequence present in the recombinant DNA, but not in the minicircle.